

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>6</sup> : C07D 471/14, 498/14, A61K 31/435, 31/55, C07D 513/14 // (C07D 471/14, 235:00, 221:00, 221:00) (C07D 471/14, 235:00, 223:00, 221:00) (C07D 471/14, 235:00, 221:00, 209:00) (C07D 498/14, 265:00, 235:00, 221:00)</p>	<p>A1</p>	<p>(11) International Publication Number: <b>WO 96/21663</b>  (43) International Publication Date: 18 July 1996 (18.07.96)</p>
<p>(21) International Application Number: PCT/US95/15828 (22) International Filing Date: 6 December 1995 (06.12.95)  (30) Priority Data: 08/371,845 12 January 1995 (12.01.95) US  (71) Applicant: MINNESOTA MINING AND MANUFACTURING COMPANY [US/US]; 3M Center, P.O. Box 33427, Saint Paul, MN 55133-3427 (US). (72) Inventor: LINDSTROM, Kyle, J.; P.O. Box 33427, Saint Paul, MN 55133-3427 (US). (74) Agents: BRINK, Robert, H. et al.; Minnesota Mining and Manufacturing Company, Office of Intellectual Property Counsel, P.O. Box 33427, Saint Paul, MN 55133-3427 (US).</p>		<p>(81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>
<p>(54) Title: IMIDAZO[4,5-c]QUINOLINE AMINES</p> <p>(57) Abstract</p> <p>Compounds and pharmaceutically acceptable salts thereof formally derived by bridging the 1- and 2-positions of 1H-imidazo[4,5-c]quinolin-4-amines of formula (II) and their use to induce interferon biosynthesis.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

## IMIDAZO[4,5-c]QUINOLINE AMINES

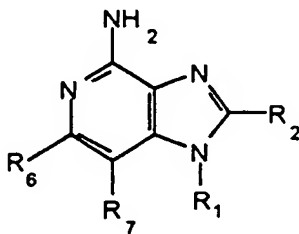
Background of the Invention5 Field of the Invention

This invention relates to imidazo[4,5-c]quinoline amine compounds. In another aspect this invention relates to immunomodulator compounds. In other aspects this invention relates to pharmaceutical compositions containing such  
10 compounds, and pharmacological methods of using such compounds.

Description of the Related Art

Certain 1H-imidazo[4,5-c]quinolin-4-amines are known as antiviral agents  
15 and/or as immunomodulators. Such compounds are disclosed, e.g., in U.S. Pat. Nos. 4,689,338, 4,929,624, 5,037,986, 5,266,575, 5,268,376, 5,346,905, European Patent Application No. 0,385,630 A2 and WO92/15582 (all to Gerster et al.). Commonly assigned copending application 08/092,002 (Lindstrom et al.) discloses  
20 immunomodulator imidazopyridine amines of the formula

20



25

R<sub>1</sub> is selected from the group consisting of hydrogen; CHR<sub>x</sub>R<sub>y</sub> wherein R<sub>x</sub> is  
30 hydrogen and R<sub>y</sub> is selected from the group consisting of straight chain, branched

- 2 -

chain, or cyclic alkyl containing one to about ten carbon atoms, straight chain or branched chain alkenyl containing two to about ten carbon atoms, straight chain or branched chain hydroxyalkyl containing one to about six carbon atoms, alkoxyalkyl wherein the alkoxy moiety contains one to about four carbon atoms and the alkyl moiety contains one to about six carbon atoms, and phenylethyl; and  $-\text{CH}=\text{CR}_2\text{R}_2$  wherein each  $\text{R}_2$  is independently straight chain, branched chain, or cyclic alkyl of one to about six carbon atoms.

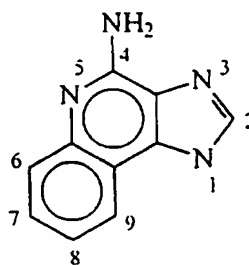
U.S. Pat. No. 5,352,784 (Nickolaides et al.) discloses immunomodulator 6,7-fused cycloalkylimidazopyridine amines.

10

### Summary of the Invention

1H-Imidazo[4,5-c]quinolin-4-amines are compounds of the general skeletal Formula I, having the numbering system shown:

15



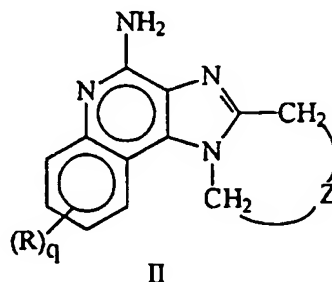
20

I

25

This invention provides compounds and pharmaceutically acceptable salts thereof formally derived by bridging the 1- and 2-positions of 1H-imidazo[4,5-c]quinolin-4-amines. In particular this invention provides compounds of Formula II

- 3 -



10 wherein R, Z, and q are as defined in detail below, and pharmaceutically acceptable salts thereof.

This invention also provides a pharmaceutical formulation comprising: (i) a compound of Formula II in an amount effective to induce interferon biosynthesis in an animal, and (ii) a pharmaceutically acceptable carrier. This invention further provides a method of inducing interferon biosynthesis in an animal, comprising the step of administering to said animal a compound of Formula II in an amount effective to induce said interferon biosynthesis.

### 20 Detailed Description of the Invention

This invention provides compounds of Formula II wherein Z is selected from the group consisting of:

-(CH<sub>2</sub>)<sub>n</sub>- wherein n is 1 to 4,

25 -(CH<sub>2</sub>)<sub>a</sub>-C(R<sub>1</sub>R<sub>2</sub>)(CH<sub>2</sub>)<sub>b</sub>-, wherein a and b are integers and a+b is 0 to 3, R<sub>1</sub> is hydrogen or alkyl of 1 to 4 carbon atoms, and R<sub>2</sub> is selected from the group consisting of alkyl of 1 to 4 carbon atoms, hydroxy, -OR<sub>3</sub> wherein R<sub>3</sub> is alkyl of 1 to 4 carbon atoms, and -NR<sub>4</sub>R'<sub>4</sub> wherein R<sub>4</sub> and R'<sub>4</sub> are independently hydrogen or alkyl of 1 to 4 carbon atoms,

- 4 -

$-(CH_2)_a-(Y)-(CH_2)_b-$  wherein a and b are integers and a+b is 0 to 3, and Y is O, S, or  $-NR_5-$  wherein  $R_5$  is hydrogen or alkyl of 1 to 4 carbon atoms,

and wherein q is 0 or 1 and R is selected from the group consisting of alkyl of 1 to 4 carbon atoms, alkoxy of 1 to 4 carbon atoms, and halogen,

5 and pharmaceutically acceptable salts thereof.

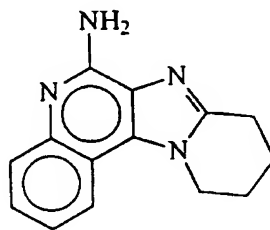
When Z is  $-(CH_2)_n-$  as defined above it is preferably alkylene having 1, 2, or 3 carbon atoms. When Z is  $-(CH_2)_3-C(R_1R_2)(CH_2)_b-$  as defined above  $R_1$  is preferably hydrogen and  $R_2$  is preferably alkyl of 1 to 4 carbon atoms, most preferably methyl. When Z is  $-(CH_2)_3-(Y)-(CH_2)_b-$  as defined above Y is preferably  
 10 O, and when Y is O a+b is preferably 1.

Preferred compounds of the invention include:

8,9,10,11-tetrahydropyrido[1',2':1,2]

imidazo[4,5-c]quinolin-6-amine, i.e.,

15



20

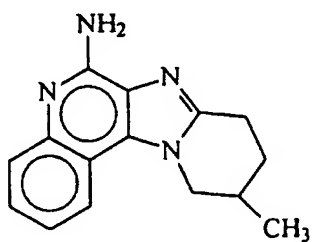
10-methyl-8,9,10,11-tetrahydropyrido[1',2':1,2]

25

imidazo[4,5-c]quinolin-6-amine, i.e.,

- 5 -

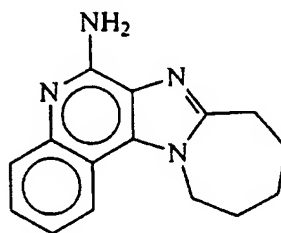
5



10

8H-9,10,11,12-tetrahydrohexamethyleneimino[1',2':1,2]  
imidazo[4,5-c]quinolin-6-amine, i.e.,

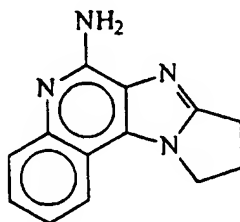
15



20

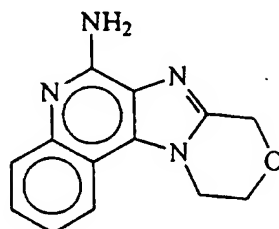
9,10-dihydro-8H-pyrrolo[1',2':1,2]  
imidazo[4,5-c]quinolin-4-amine, i.e.,

25



- 6 -

and 10,11-dihydro-8H-[1,4]-oxazino[4',3':1,2]  
imidazo[4,5-c]quinolin-6-amine, i.e.,



Reaction Scheme I illustrates preparation of the compounds of the invention. The unsubstituted compound of Formula XI is a known commercially available compound and other compounds of Formula XI can be prepared by methods known to those skilled in the art and disclosed, e.g., in Chem. Ber. 1927, 60, 1108 (Kohler) and J. Heterocyclic Chem. 1988, 25, 857 (Kappe).

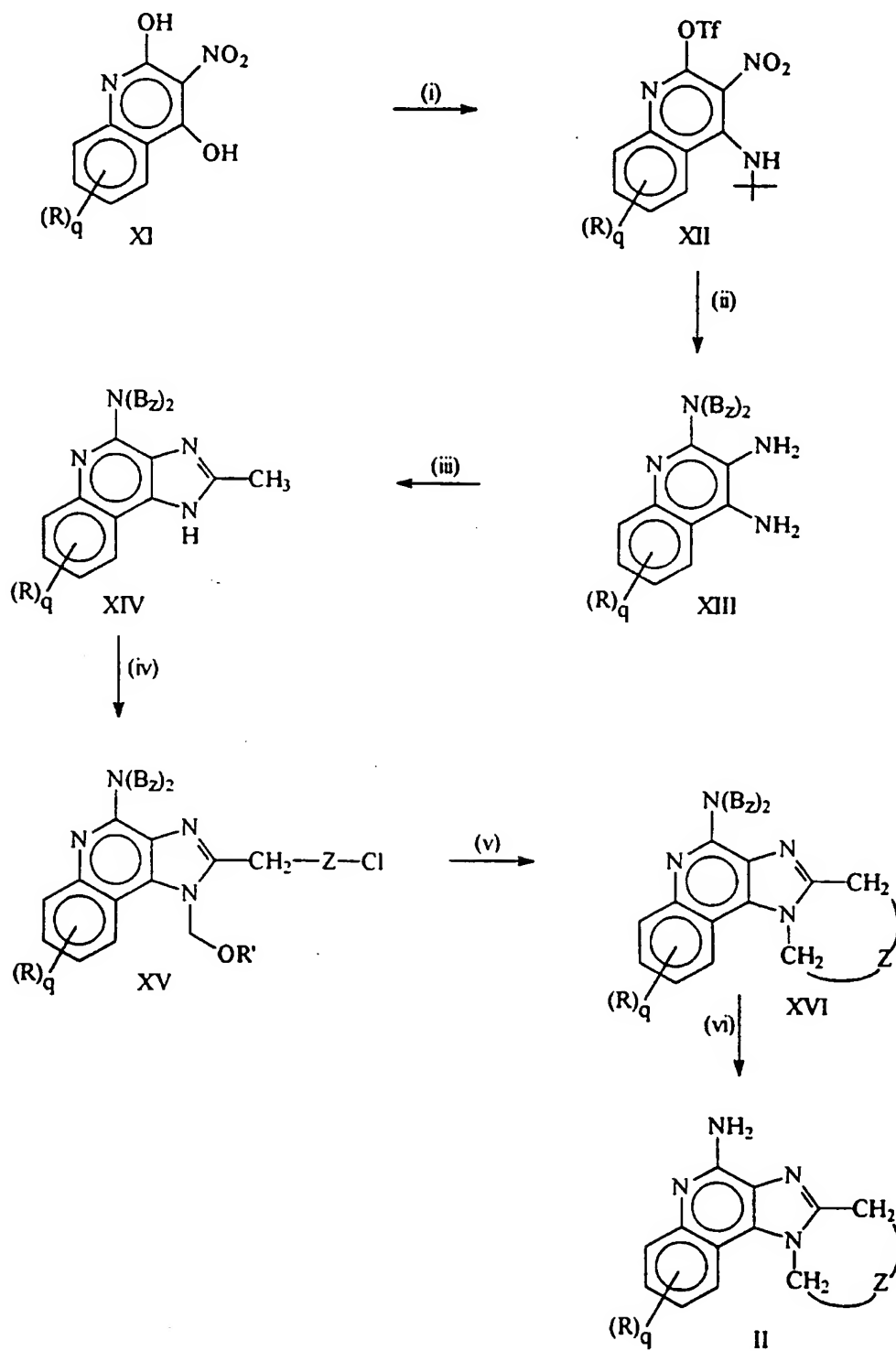
In step (i) a 3-nitroquinoline-2,4-disulfonate is first prepared by reacting a 2,4-dihydroxy-3-nitroquinoline with a sulfonyl halide or preferably a sulfonic anhydride. Suitable sulfonyl halides include alkylsulfonyl halides such as methanesulfonyl chloride and trifluoromethanesulfonyl chloride, and arylsulfonyl halides such as benzenesulfonyl chloride, p-bromobenzenesulfonyl chloride, and p-toluenesulfonyl chloride. Suitable sulfonic anhydrides include those corresponding to the above-mentioned sulfonyl halides. A particularly preferred sulfonic anhydride is trifluoromethanesulfonic anhydride.

Reaction conditions preferably involve first combining a compound of Formula XI with a base, preferably an excess of a tertiary amine base (e.g., a trialkylamine base such as triethylamine) and preferably in an appropriate solvent such as dichloromethane and then adding the sulfonyl halide or the sulfonic anhydride. The addition is preferably carried out in a controlled fashion (e.g., dropwise) and at a reduced temperature (e.g., at about 0°C).



- 7 -

## REACTION SCHEME I



- 8 -

The disulfonate is then reacted with tert-butylamine, preferably in the presence of an excess of a tertiary amine base in a solvent such as dichloromethane to afford a compound of Formula XII. The reaction can be carried out by adding the tertiary amine base to the reaction mixture resulting from the first portion of

5 step (i), cooling to a reduced temperature (e.g., 0°C), and adding the tert-butylamine in a controlled fashion (e.g., dropwise). The reaction can also be carried out by adding the tert-butylamine to a solution of the disulfonate and a tertiary amine base in a solvent such as dichloromethane. The reaction can be run at relatively low temperatures, e.g., about 0°C, in order to decrease the amount of

10 undesired 2-aminated and 2,4-diaminated side products. It is sometimes necessary or desirable to heat the reaction mixture after the addition in order to complete the reaction.

In step (ii) the compound of Formula XII is reacted with dibenzylamine. The reaction can be carried out by placing the starting material and the

15 dibenzylamine in an inert solvent such as benzene, toluene, or xylene, and heating at a temperature and for a time sufficient to cause displacement of the sulfonate group by the dibenzylamine, such temperature and time being readily selected by those skilled in the art. The tert-butyl group is then removed by heating in a polar solvent such as methanol in the presence of an acid such as hydrochloric acid.

20 The nitro group is then reduced to an amino group. Methods for such a reduction are well known to those skilled in the art. A preferred method involves in situ generation of  $\text{Ni}_2\text{B}$  from sodium borohydride and  $\text{NiCl}_2$  in methanol to afford a reducing agent solution. The nitro compound is added to the reducing agent solution to effect reduction of the nitro group. The product is a compound of

25 Formula XIII.

In step (iii) a compound of Formula XIII is reacted with acetic acid or an equivalent thereof to afford the cyclized compound of Formula XIV. Suitable equivalents to acetic acid include corresponding acetyl halides and orthoesters. When using acetic acid or an orthoester equivalent the reaction can be run in the

30 absence of solvent or in an inert solvent such as xylene or toluene with sufficient

- 9 -

heating (e.g., at about 80-150°C depending on the solvent if any) to drive off any alcohol or water formed as a side product of the reaction. When using an acetyl halide the reaction is preferably run in acetic acid with heating.

In step (iv) the cyclized compound of Formula XIV is first substituted at the  
5 1-position with an alkoxymethyl group ( $-\text{CH}_2\text{OR}'$  wherein  $\text{R}'$  is alkyl such as ethyl) in order to protect the 1-nitrogen. The substitution can be carried out by treating the compound of Formula XIV with sodium hydride and a halomethylalkyl ether such as chloromethyl ethyl ether. The resulting 1-protected compound is then substituted on the 2-methyl group with a moiety of the formula  $-\text{ZCl}$  to afford a  
10 compound of Formula XV. This can be accomplished by metalating the 2-methyl group of the 1-protected compound, e.g., by treating with *n*-butyl lithium in tetrahydrofuran, then adding an alkylating agent of the formula  $\text{X}'\text{-ZCl}$  wherein  $\text{X}'$  is a more facile leaving group than chloro (e.g.,  $\text{X}'$  can be bromo). Another suitable method of substituting on the 2-methyl group involves metalating, reacting with an  
15 epoxide such as ethylene oxide, and converting the resulting 2-(hydroxyalkyl) compound to the corresponding halide by reacting with a chlorinating agent such as thionyl chloride in an inert solvent.

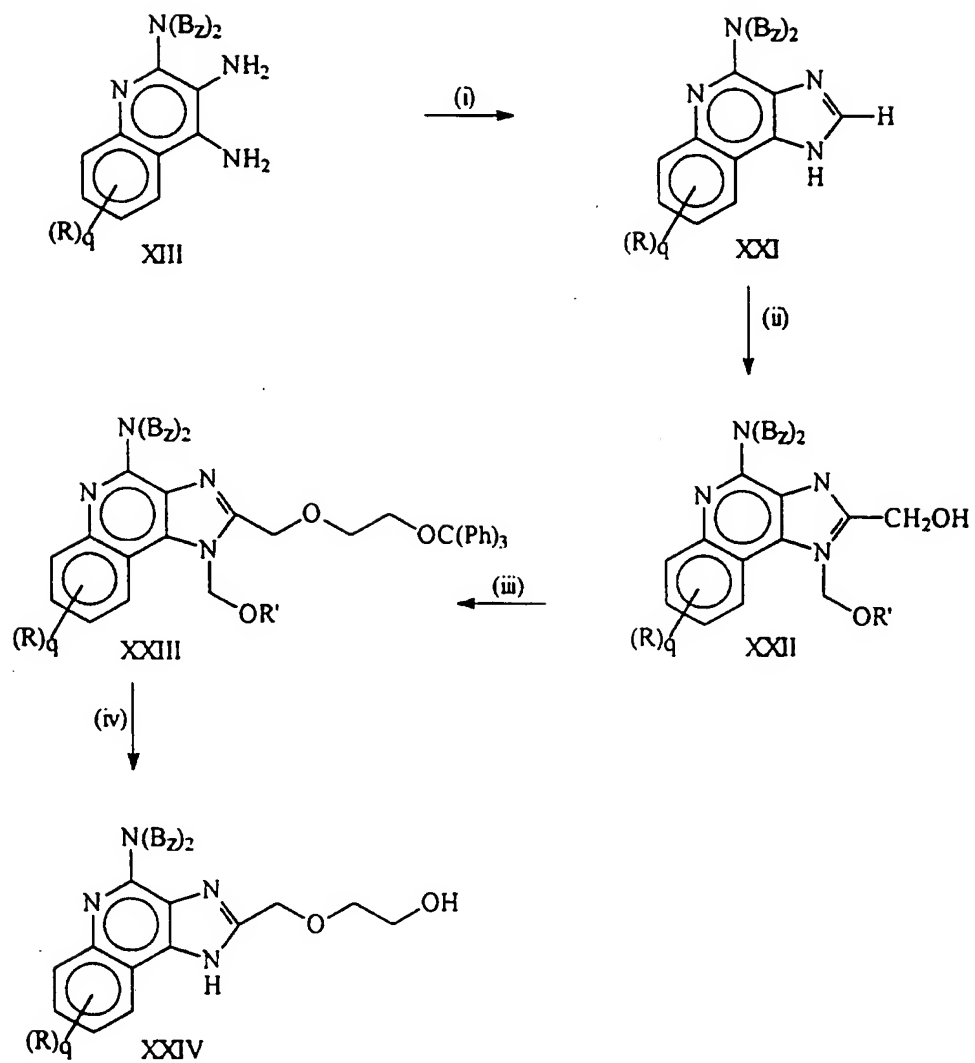
In step (v) the 1-position of a compound of Formula XV is deprotected, e.g., by heating in the presence of  $\text{HCl}$  in a solvent such as methanol. The resulting  
20 1-hydrogen compound is then cyclized by formal displacement of the chloro group by the 1-nitrogen. Treating with sodium iodide in a polar solvent such as acetone in the presence of a neutralizing base (e.g., potassium carbonate) is a suitable means for affecting cyclization. A compound of Formula XVI results.

In step (vi) the compound of Formula XVI is hydrogenolyzed to afford the  
25 corresponding 4-amino compound of Formula II. Conventional well known catalytic hydrogenation conditions are suitable. Preferred conditions involve heating in formic acid in the presence of  $\text{Pd}(\text{OH})_2/\text{C}$ .

Reaction Scheme II shows a route to certain compounds of the invention not amenable to preparation via Reaction Scheme I.

30

## REACTION SCHEME II



In step (i) a compound of Formula XIII is cyclized using formic acid or an equivalent thereof such as an orthoformate (e.g., triethylorthoformate) to provide a compound of Formula XXI (see, e.g., step (iii) of Reaction Scheme I). In step (ii) the compound of Formula XXI is protected at the 1-position as described above in connection with step (iv) of Reaction Scheme I. The protected product is then formylated at the 2-position by first metalating in a polar solvent such as tetrahydrofuran, e.g., reacting with n-butyllithium, and then treating with formaldehyde. Step (iii) involves homologating the 2-substituent of the compound of Formula XXII, e.g., by reacting with 1-bromo-2-(triphenylmethoxy)ethane. The product of Formula XXIII can be carried on to a compound of Formula II by acid catalyzed hydrolysis of the protecting groups to afford a compound of Formula XXIV, conversion of the hydroxyl group on the 2-substituent to an appropriate leaving group, cyclization (e.g., as described above in connection with step (v) of Reaction Scheme I), and reductive cleavage as described above in connection with step (vi) of Reaction Scheme I.

Compounds of Formula II not amenable to being prepared via the routes illustrated in Reaction Schemes I or II, or otherwise described herein can be prepared using variants of the illustrated schemes involving well known synthetic alternatives, alteration of the order of steps, and the like.

The product compound of Formula II can be isolated by the conventional means disclosed in U.S. Pat. No. 4,689,338 (Gerster), such as, for example, removal of the solvent and recrystallization from an appropriate solvent (e.g., N,N-dimethylformamide) or solvent mixture, or by dissolution in an appropriate solvent (such as methanol) and re-precipitation by addition of a second solvent in which the compound is insoluble.

A compound of Formula II can be used as an antiviral agent itself or it can be used in the form of a pharmaceutically acceptable salt such as a hydrochloride, dihydrogen sulfate, trihydrogen phosphate, hydrogen nitrate, methanesulfonate or a salt of another pharmaceutically acceptable acid. A pharmaceutically acceptable salt of a compound of Formula II can be readily prepared, generally by reaction of the

compound with an equimolar amount of a relatively strong acid, preferably an inorganic acid such as hydrochloric, sulfuric, or phosphoric acid, or an organic acid such as methanesulfonic acid, in a polar solvent. Isolation of the salt is facilitated by the addition of a solvent, such as diethyl ether, in which the salt is insoluble.

- 5           A compound of the invention can be formulated for the various routes of administration (e.g., oral administration by tablet, capsule, oral suspension, or the like, topical, transdermal, or parenteral) by combining a therapeutically effective amount of a compound of Formula II with an appropriate pharmaceutically acceptable vehicle including any adjuvants and excipients suitable for the selected
- 10 dosage form. Suitable formulations include parenteral solutions, topical creams, gels, and ointments, and oral tablets and capsules. Methods of manufacture of such pharmaceutical compositions are well known to those skilled in the art and disclosed, e.g., in Remington's Pharmaceutical Sciences, 18th Edition, 1990 Mack Publishing Company, A. R. Gennaro, Editor. Consequently, particular formulations
- 15 suitable for a selected route of administration can be readily identified and prepared by those skilled in the art. A solid dosage form, for example, contains a compound of Formula II, and one or more diluents (e.g., dicalcium phosphate, calcium sulfate, lactose, mannitol, cellulose, kaolin, sodium chloride, starch, sucrose, inositol, sorbitol), binders (e.g., starch, gelatin, sucrose, glucose, dextrose, molasses,
- 20 lactose, natural and synthetic gums), lubricants (e.g., talc, magnesium stearate, calcium stearate, stearic acid, hydrogenated vegetable oils, polyethylene glycols), disintegrants (e.g., corn starch, potato starch, clays, cellulose, alginates), coloring agents, and flavoring agents. A parenteral solution contains a compound of Formula II and a pharmaceutically acceptable aqueous vehicle including suitable
- 25 excipients, such as acids (hydrochloric acid, lactic acid, acetic acid, aspartic acid or mixtures thereof) or bases (sodium hydroxide) sufficient to achieve a pH of 2 to about 6, and tonicity adjusters (e.g., sorbitol or glycerin) in order that the formulation is isotonic with serum. Topical or transdermal formulations contain a compound of Formula II in a cream, ointment, or a pressure sensitive adhesive
- 30 composition. A cream can contain emollients (e.g., cetyl alcohol, stearyl alcohol,

- 13 -

petrolatum, light mineral oil, acetylated lanolin), emulsifiers (e.g., nonionic surfactants such as polysorbate 60, sorbitan monostearate), thickeners (e.g., montmorillonite clays or long chain alcohols such as cetearyl alcohol, cetyl alcohol, and stearyl alcohol), and preservatives (e.g., methylparaben, propylparaben, benzyl alcohol) in amounts readily selected by those skilled in the art. An ointment contains an ointment base (e.g., polyethylene glycol, petrolatum) and emollients and thickeners.

The amount of compound of Formula II that constitutes a therapeutically effective amount will vary according to the particular compound used, the desired therapeutic effect, the condition being treated, the dosing regimen, and the route of administration. Generally, a compound of Formula II will be present in a parenteral formulation in an amount of about 0.1 to about 10 percent by weight based on the total weight of the formulation. Similarly an oral tablet or capsule will generally contain about 0.5 to about 50 percent by weight; and a topical or transdermal formulation will contain about 0.1 to about 10 percent by weight. Particular formulations will be easily selected by those skilled in the art.

A number of compounds of Formula II were tested and found to induce biosynthesis of interferon in human cells and in mice. These results suggest that at least certain compounds of the invention might be useful in treating viral diseases (e.g., hepatitis, herpes, warts) and diseases such as rheumatoid arthritis, eczema, psoriasis, multiple sclerosis, essential thrombocythaemia, cancer such as basal cell carcinoma, and other neoplastic diseases.

The examples below are intended to illustrate the invention. The structures were confirmed by nuclear magnetic resonance spectroscopy.

- 14 -

## Example 1

8,9,10,11-Tetrahydropyrido[1',2':1,2]  
imidazo[4,5-c]quinolin-6-amine

5 Part A

Triethylamine (84 mL, 0.6 mole) was added to a suspension of 3-nitro-2,4-quinolinediol (40 g, 0.194 mole) in methylene chloride (1200 mL). The resulting solution was cooled in an ice bath and trifluoromethanesulfonic anhydride (67.2 mL, 0.40 mole) was added. After the addition was complete, the reaction was  
10 heated on a steam bath for 10 minutes then once again cooled in an ice bath. *Tert*-butylamine (42 mL, 0.4 mole) was added then the reaction was heated on a steam bath for 15 minutes. The reaction mixture was washed with aqueous sodium bicarbonate (500 mL), dried over magnesium sulfate then concentrated under vacuum. The concentrate was put through a layer of silica gel and the silica gel was  
15 eluted with methylene chloride. The methylene chloride solution was evaporated under vacuum to provide 54 g of [4-(1,1-dimethylethyl)amino-3-nitroquinolin-2-yl] trifluoromethanesulfonate.

Part B

Triethylamine (19.2 mL, 0.137 mole) was added to a solution of [4-(1,1-dimethylethyl)amino-3-nitroquinolin-2-yl] trifluoromethanesulfonate (54 g, 0.137 mole) in toluene (about 1 L). Dibenzylamine (27 mL, 0.137 mole) was added and the reaction mixture was heated at reflux for about 2 hours. The reaction mixture was concentrated under vacuum. The residue was diluted with methanol (900 mL).  
Hydrochloric acid (100 mL of 6N) was added and the reaction mixture was heated  
25 at reflux for 1 hour. The reaction mixture was stirred at ambient temperature overnight. The resulting precipitate was isolated by filtration, washed with methanol and then dried to provide 42.1 g of N<sup>2</sup>,N<sup>2</sup>-bis(phenylmethyl)-3-nitroquinoline-2,4-diamine hydrochloride as a yellow solid.



- 15 -

Part C

Sodium borohydride (5.5 g, 0.147 mmole) was carefully added to a solution containing nickel (II) chloride hydrate (11.9 g, 0.05 mole) in methanol (1200 mL). N<sup>2</sup>,N<sup>2</sup>-bis(phenylmethyl)-3-nitroquinoline-2,4-diamine hydrochloride (42.1 g, 0.1 mole) was taken up in a mixture of methylene chloride (400 mL) and methanol (200 mL) and added to the nickel borate reagent. Additional sodium borohydride was carefully added until the generated foam was colorless. The reaction mixture was filtered through a layer of Celite<sup>TM</sup> filter agent. The filtrate was concentrated under vacuum. The residue was partitioned between methylene chloride and water. The methylene chloride layer was dried over magnesium sulfate then concentrated under vacuum. The residue was taken up in diethyl ether (about 1200 mL). Hydrochloric acid was bubbled through the ether solution for 5 minutes. The resulting precipitate was collected and dried to provide 32 g of N<sup>2</sup>,N<sup>2</sup>-bis(phenylmethyl)quinoline-2,3,4-triamine hydrochloride.

Part D

Triethylamine (3.6 mL, 25.6 mmole) was added to a suspension of N<sup>2</sup>,N<sup>2</sup>-bis(phenylmethyl)quinoline-2,3,4-triamine hydrochloride (5 g, 12.8 mmoles) in acetic acid (120 mL). Acetyl chloride (0.91 mL, 12.8 mmole) was added and the reaction mixture was heated at reflux for 5 to 6 hours then concentrated under vacuum. The residue was partitioned between diethyl ether and saturated aqueous sodium bicarbonate. The ether layer was dried then concentrated under vacuum. The residue was purified by column chromatography (silica gel eluting with methylene chloride containing 1-5% v/v ethyl acetate) to provide about 2.4 g of N,N-bis(phenylmethyl)-2-methyl-1H-imidazo[4,5-c]quinolin-4-amine.

Part E

A suspension of sodium hydride (0.064 g, 2 mmole) in tetrahydrofuran (15 mL) was cooled in an ice bath. N,N-bis(phenylmethyl)-2-methyl-1H-imidazo[4,5-c]quinolin-4-amine (0.5 g, 1.32 mmole) was added and the reaction mixture was allowed to warm to room temperature for 20 minutes. Chloromethyl ethyl ether was added and the reaction mixture was stirred for 30 minutes. The reaction

- 16 -

mixture was diluted with diethyl ether, washed twice with water, dried over magnesium sulfate and then concentrated under vacuum. The residue was purified by column chromatography (silica gel eluting with methylene chloride) to provide 0.43 g of N,N-bis(phenylmethyl)-1-ethoxymethyl-2-methyl-1H-imidazo[4,5-c]quinolin-4-amine.

#### Part F

Under a nitrogen atmosphere, a solution of N,N-bis(phenylmethyl)-1-ethoxymethyl-2-methyl-1H-imidazo[4,5-c]quinolin-4-amine (1.12 g, 2.56 mmole) in tetrahydrofuran (20 mL) was cooled to -78°C. Butyllithium (1.03 mL of 2.5 M in hexanes, 2.56 mmole) was added and the reaction mixture was stirred for 5 minutes. 1-Bromo-3-chloropropane (2.7 mL, 25 mmole) was added and the reaction mixture was allowed to warm to ambient temperature. When the reaction was complete, as indicated by thin layer chromatography (silica gel, 30% ethyl acetate in hexanes v/v), the reaction mixture was diluted with diethyl ether and water. The ether layer was separated, dried over magnesium sulfate and then concentrated under vacuum. The resulting residue was purified by column chromatography (silica gel eluting with 10-20% ethyl acetate in hexanes v/v) to provide 0.76 g of N,N-bis(phenylmethyl)-2-(4-chlorobutyl)-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-4-amine.

#### Part G

Methanol (several mL) was added to a suspension of N,N-bis(phenylmethyl)-2-(4-chlorobutyl)-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-4-amine (0.76 g, 1.5 mmole) in 3N hydrochloric acid (20 mL). The reaction mixture was heated on a steam bath for 4 hours then partitioned between methylene chloride and aqueous saturated sodium bicarbonate. The methylene chloride layer was separated, dried over magnesium sulfate and then concentrated under vacuum to provide 0.64 g of N,N-bis(phenylmethyl)-2-(4-chlorobutyl)-1H-imidazo[4,5-c]quinolin-4-amine.

**Part H**

Sodium iodide (1 g, 6 mmole) and potassium carbonate (0.8 g, 6 mmole) were added to a solution of N,N-bis(phenylmethyl)-2-(4-chlorobutyl)-1H-imidazo[4,5-c]quinolin-4-amine (0.55 g, 1.2 mmole) in acetone. The reaction mixture was heated at reflux for 4 hours, filtered, and then concentrated under vacuum. The residue was purified by flash chromatography (silica gel eluting with 10-15% ethyl acetate in hexanes v/v). Nuclear magnetic resonance spectroscopy indicated the possible presence of iodo intermediate; so the residue was taken up in acetone, combined with sodium iodide and potassium carbonate and heated at reflux over night. The reaction was worked up to provide 0.38 g of N,N-bis(phenylmethyl)-8,9,10,11 tetrahydropyrido-[1',2':1,2]imidazo[4,5-c]quinolin-4-amine.

**Part I**

Palladium hydroxide on carbon (0.35 g) was added to a solution of N,N-bis(phenylmethyl)-8,9,10,11 tetrahydropyrido[1',2':1,2]imidazo[4,5-c]quinolin-4-amine (0.38 g, 0.9 mmole) in formic acid (about 15 mL). The reaction mixture was heated at reflux for 3 days then diluted with methylene chloride and made basic (about pH 9) with 10% sodium hydroxide. The methylene chloride layer was separated, dried over magnesium sulfate and then concentrated under vacuum to provide a white solid. The material was purified by column chromatography (silica gel eluting with 4-10% methanol in methylene chloride v/v) to provide a solid. The solid was suspended in methylene chloride (20 mL) then isolated by filtration and dried to provide 70 mg of 8,9,10,11 tetrahydropyrido[1',2':1,2]imidazo[4,5-c]quinolin-4-amine as a solid, m.p. 275-278°C. Calculated for  $C_{14}H_{14}N_4 + 0.2 CH_2Cl_2$ : %C, 66.81; %H, 5.69; %N, 21.95; Found: %C, 67.06; %H, 5.60; %N, 22.18.

- 18 -

## Example 2

10-Methyl-8,9,10,11-tetrahydropyrido[1',2':1,2]  
imidazo[4,5-c]quinolin-6-amine

Part A

5           A solution of N,N-bis(phenylmethyl)-1-ethoxymethyl-2-methyl-1H-imidazo[4,5-c]quinolin-4-amine (1.8 g, 4.12 mmole, Example 1 Part E) in tetrahydrofuran (40 mL) was cooled to -78°C. Butyllithium (1.7 mL of 2.5 M in hexanes, 4.2 mmole) was added and the reaction mixture was stirred for 20 minutes. 1-Bromo-3-chloro-2-methylpropane (4.8 mL, 41 mmole) was added and  
10          the reaction mixture was allowed to warm to ambient temperature over a period of 1 hour. The reaction mixture was diluted with diethyl ether and water. The ether layer was separated, dried over magnesium sulfate and then concentrated under vacuum. The resulting residue was purified by flash chromatography (silica gel eluting with 5-20% ethyl acetate in hexanes v/v) to provide 0.65 g of N,N-  
15          bis(phenylmethyl)-2-(4-chloro-3-methylbutyl)-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-4-amine.

Part B

          Hydrochloric acid (80 mL of 6N) was added to a suspension of N,N-bis(phenylmethyl)-2-(4-chloro-3-methylbutyl)-1-ethoxymethyl-1H-imidazo[4,5-  
20          c]quinolin-4-amine (0.64 g, 1.2 mmole) in methanol (40 mL). The reaction mixture was heated at reflux for 2 hours (All of the methanol was driven off during this period.), diluted with methylene chloride and then made basic with 10% sodium hydroxide. The methylene chloride layer was separated, dried over magnesium sulfate and then concentrated to provide about 0.5 g of N,N-bis(phenylmethyl)-2-  
25          (4-chloro-3-methylbutyl)-1H-imidazo[4,5-c]quinolin-4-amine.

Part C

          A large excess (about 10 fold) of both sodium iodide and potassium carbonate were added to a solution of N,N-bis(phenylmethyl)-2-(4-chloro-3-methylbutyl)-1H-imidazo[4,5-c]quinolin-4-amine (about 0.5 g) in acetone (about 75  
30          mL). The reaction mixture was heated at reflux overnight, diluted with additional

- 19 -

acetone, filtered and then concentrated under vacuum. The residue was washed with methylene chloride to recover the product from salts then purified by column chromatography (silica gel eluting with 3% ethyl acetate in methylene chloride v/v) to provide 0.35 g of N,N-bis(phenylmethyl)-8,9,10,11 tetrahydro-10-methylpyrido[1',2':1,2]imidazo[4,5-c]quinolin-4-amine.

#### Part D

Palladium hydroxide on carbon (0.35 g) was added to a solution of N,N-bis(phenylmethyl)-8,9,10,11 tetrahydro-10-methylpyrido[1',2':1,2]imidazo[4,5-c]quinolin-4-amine (0.35 g, 0.809 mmole) in formic acid (about 15 mL). The reaction mixture was heated at reflux for 3 days, diluted with a mixture of methanol and water, and then filtered through Celite<sup>TM</sup> filter agent. The filtrate was concentrated under vacuum and then made basic with 10% sodium hydroxide. The resulting precipitate was isolated by filtration then purified by column chromatography (silica gel eluting with 2-5% methanol in methylene chloride v/v) to provide 0.1 g of 10-methyl-8,9,10,11-tetrahydropyrido[1',2':1,2]imidazo[4,5-c]quinolin-6-amine as a solid, m.p. 279-281°C. Calculated for C<sub>15</sub>H<sub>16</sub>N<sub>4</sub>: %C, 71.40; %H, 6.39; %N, 22.20; Found: %C, 71.10; %H, 6.46; %N, 22.25.

#### Example 3

8H-9,10,11,12-Tetrahydrohexamethyleneimino[1',2':1,2]imidazo[4,5-c]quinolin-6-amine Hydrate

#### Part A

A solution of N,N-bis(phenylmethyl)-1-ethoxymethyl-2-methyl-1H-imidazo[4,5-c]quinolin-4-amine (1.5 g, 3.43 mmole, Example 1 Part E) in tetrahydrofuran (30 mL) was cooled to -78°C. Butyllithium (1.4 mL of 2.5 M in hexanes, 3.5 mmole) was added dropwise followed by the addition of 1-bromo-4-chlorobutane (4 mL, 34 mmole). The reaction mixture was allowed to warm to ambient temperature then it was quenched with diethyl ether and water. The ether layer was separated, dried over magnesium sulfate and then concentrated under vacuum. The resulting residue was purified by column chromatography (silica gel

- 20 -

eluting with 10% ethyl acetate in hexanes v/v) to provide 1.5 g of N,N-bis(phenylmethyl)-2-(5-chloropentyl)-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-4-amine.

#### Part B

5 A suspension of N,N-bis(phenylmethyl)-2-(5-chloropentyl)-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-4-amine (1.5 g, 3 mmole) in 6N hydrochloric acid (100 mL) was heated at reflux for 1 hour. The reaction mixture was cooled, diluted with methylene chloride, made basic with 10% sodium hydroxide and then extracted with methylene chloride (400 mL total). The methylene chloride extracts were  
10 combined, dried over magnesium sulfate and then concentrated under vacuum to provide about 1.3 g of N,N-bis(phenylmethyl)-2-(5-chloropentyl)-1H-imidazo[4,5-c]quinolin-4-amine.

#### Part C

Using the method of Example 2 Part C, N,N-bis(phenylmethyl)-2-(5-chloropentyl)-1H-imidazo[4,5-c]quinolin-4-amine (1.3 g, 3 mmole) was cyclized to  
15 provide 1.1 g of N,N-bis(phenylmethyl)-8H-9,10,11,12-tetrahydrohexamethyleneimino[1',2':1,2]imidazo[4,5-c]quinolin-6-amine as a white solid.

#### Part D

20 Palladium hydroxide on carbon (1 g) was added to a solution of N,N-bis(phenylmethyl)-8H-9,10,11,12-tetrahydrohexamethyleneimino[1',2':1,2]imidazo[4,5-c]quinolin-6-amine (1 g, 2.3 mmole) in formic acid (about 30 mL). The reaction mixture was heated at reflux for 4 days, filtered, washed with methanol/methylene chloride and then  
25 concentrated under vacuum. The residue was partitioned between methylene chloride and 10% sodium hydroxide. The methylene chloride layer was separated, dried over magnesium sulfate and then concentrated under vacuum. The residue was purified by column chromatography (silica gel eluting with 3-10% methanol in methylene chloride v/v) to provide 0.4 g of 8H-9,10,11,12-  
30 tetrahydrohexamethyleneimino[1',2':1,2]imidazo[4,5-c]quinolin-6-amine hydrate as

- 21 -

a white solid, m.p. 237-240°C. Calculated for  $C_{15}H_{16}N_4 + 1/3 H_2O$ : %C, 69.74; %H, 6.50; %N, 21.69; Found: %C, 69.74; %H, 6.27; %N, 21.37.

## Example 4

5                    9,10-Dihydro-8H-pyrrolo[1',2':1,2]  
                    imidazo[4,5-c]quinolin-4-amine Hydrate

Part A

A solution of N,N-bis(phenylmethyl)-1-ethoxymethyl-2-methyl-1H-imidazo[4,5-c]quinolin-4-amine (1.7 g, 3.9 mmole, Example 1 Part E) in  
10 tetrahydrofuran (50 mL) was cooled to -78°C. Butyllithium (1.6 mL of 2.5 M in hexanes, 4.1 mmole) was added dropwise and the reaction mixture was stirred for 5 minutes. Ethylene oxide was run over the surface of the reaction mixture. After 10 minutes the reaction mixture was allowed to warm to ambient temperature. The ethylene oxide addition was stopped when the reaction temperature reached 0°C.  
15 The reaction was quenched with diethyl ether and water. The ether layer was separated, dried over magnesium sulfate and then concentrated under vacuum. The resulting residue was purified by column chromatography (silica gel eluting with 5-10% ethyl acetate in methylene chloride v/v) to provide 1.4 g of N,N-bis(phenylmethyl)-2-(3-hydroxypropyl)-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-  
20 4-amine.

Part B

Thionyl chloride (5 mL, 68 mmole) was added to N,N-bis(phenylmethyl)-2-(3-hydroxypropyl)-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-4-amine (1 g, 2.1 mmole) and the reaction mixture was stirred rapidly until thin layer chromatography  
25 (silica gel, 10% ethyl acetate in methylene chloride v/v) indicated that the reaction was complete. The reaction mixture was diluted with methylene chloride then neutralized with 10% sodium hydroxide and sodium bicarbonate. The methylene chloride layer was separated, dried over magnesium sulfate and then concentrated under vacuum. The residue was purified by column chromatography (silica gel  
30 eluting with 10-30% ethyl acetate in hexanes v/v) to provide 1 g of N,N-

- 22 -

bis(phenylmethyl)-2-(3-chloropropyl)-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-4-amine.

#### Part C

A suspension of N,N-bis(phenylmethyl)-2-(3-chloropropyl)-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-4-amine (1 g, 2.0 mmole) in 6N hydrochloric acid (80 mL) was heated at reflux for 2 hours and then stirred at ambient temperature overnight. The reaction mixture was neutralized with 10% sodium hydroxide then extracted with methylene chloride. The extract was dried over magnesium sulfate then concentrated under vacuum to provide 0.8 g of N,N-bis(phenylmethyl)-2-(3-chloropropyl)-1H-imidazo[4,5-c]quinolin-4-amine.

#### Part D

Potassium carbonate (10X excess) and sodium iodide (5X excess) were added to a solution of N,N-bis(phenylmethyl)-2-(3-chloropropyl)-1H-imidazo[4,5-c]quinolin-4-amine (0.8 g, 1.8 mmole) in acetone. The reaction was heated at reflux for 2 hours, filtered and then concentrated under vacuum. The residue was partitioned between methylene chloride and water. The methylene chloride layer was separated, dried over magnesium sulfate and then concentrated under vacuum. The residue was purified by column chromatography (silica gel eluting with 10-30% ethyl acetate in hexanes v/v) to provide 0.25 g of N,N-bis(phenylmethyl)-9,10-dihydro-8H-pyrrolo[1',2':1,2]imidazo[4,5-c]quinolin-4-amine.

#### Part E

Palladium hydroxide on carbon (0.5 g) was added to a solution of N,N-bis(phenylmethyl)-9,10-dihydro-8H-pyrrolo[1',2':1,2]imidazo[4,5-c]quinolin-4-amine (0.25 g, 0.62 mmole) in formic acid (75 mL). The reaction mixture was heated at reflux for 4 days then diluted with methanol and filtered. The filtrate was concentrated under vacuum then mixed with water and sodium bicarbonate. A gray precipitate was isolated by filtration. The filtrate was concentrated. The resulting residue was slurried with a mixture of methanol and methylene chloride and then filtered. The filtrate was combined with the previously isolated gray precipitate then purified by column chromatography (silica gel eluting with 3-5% methanol in



- 23 -

methylene chloride v/v) to provide 80 mg of N,N-bis(phenylmethyl)-9,10-dihydro-8H-pyrrolo[1',2':1,2]imidazo[4,5-c]quinolin-4-amine as a white solid, m.p. 275-277°C. Analysis: Calculated for  $C_{13}H_{12}N_4 + 1/3 H_2O$ : %C, 67.81; %H, 5.54; %N, 24.33; Found: %C, 67.80; %H, 5.26; %N, 24.28.

5

## Example 5

10,11-Dihydro-8H-[1,4]-oxazino[4',3':1,2]  
imidazo[4,5-c]quinolin-6-amine

Part A

- 10 A suspension of N<sup>2</sup>,N<sup>2</sup>-bis(phenylmethyl)quinoline-2,3,4-triamine hydrochloride (10 g, 25.6 mmole, Example 1 Part C) in triethyl orthoformate (40 mL) was heated at about 120°C for 30 minutes, cooled to ambient temperature and then diluted with diethyl ether. The resulting precipitate was isolated by filtration then partitioned between ammonium hydroxide and methylene chloride. The
- 15 methylene chloride layer was separated, washed twice with water, dried over magnesium sulfate and then concentrated under vacuum to provide 8.6 g of N,N-bis(phenylmethyl)-1H-imidazo[4,5-c]quinolin-4-amine as a dark brown solid.

Part B

- 20 A solution of N,N-bis(phenylmethyl)-1H-imidazo[4,5-c]quinolin-4-amine (2 g, 5.49 mmole) in tetrahydrofuran (10 mL) was added to a suspension of sodium hydride (0.21 g, 6.58 mmole) in tetrahydrofuran (25 mL). After 30 minutes chloromethyl ethyl ether (0.61 mL, 6.58 mmole) was added and the reaction mixture was stirred for 2 hours. The reaction mixture was diluted with diethyl ether, washed with water, dried over magnesium sulfate, and then concentrated
- 25 under vacuum to provide crude product as a brown oil. The oil was purified by column chromatography (silica gel eluting with 20-30% ethyl acetate in hexanes v/v) to provide 1.77 g of N,N-bis(phenylmethyl)-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-4-amine as a white/tan solid.

Part C

Butyllithium (1.6 mL of 2.5 M in hexanes, 4 mmole) was added to a chilled (dry ice/acetone bath) solution of N,N-bis(phenylmethyl)-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-4-amine (1.7 g, 4 mmole) in tetrahydrofuran. No color change was observed. The reaction mixture was warmed to -20°C (dry ice/carbon tetrachloride) and the reaction turned red in color. Formaldehyde gas entrained in a flow of nitrogen was added to the reaction mixture. After several minutes the reaction turned to a solid mass and the ice bath was removed. The reaction mixture was allowed to warm to ambient temperature and the color of the reaction mixture changed from red to yellow. The reaction was diluted with diethyl ether and water. The ether layer was separated, dried over magnesium sulfate and then concentrated under vacuum. The residue was purified by column chromatography (silica gel eluting with 10-20% ethyl acetate in hexanes v/v) to provide 1 g of 4-bis(phenylmethyl)amino-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-2-methanol.

15 Part D

Sodium hydride (0.11 g, 3.3 mmole) was added to a solution of 4-bis(phenylmethyl)amino-1-ethoxymethyl-1H-imidazo[4,5-c]quinolin-2-methanol (1 g, 2.21 mmole) in N,N-dimethylformamide (15 mL) and the resulting mixture was stirred for 10 minutes. 1-Bromo-2-(trityloxy)ethane was added and stirring was continued at ambient temperature for 3-4 hours. The reaction was quenched with diethyl ether and water. The ether layer was separated, washed several times with water, dried over magnesium sulfate and then concentrated under vacuum. The residue was purified by column chromatography (silica gel eluting with 10-30% ethyl acetate in hexanes v/v) to provide 1.1 g of N,N-bis(phenylmethyl)-1-ethoxymethyl-2-[(2-triphenylmethoxy)ethoxy]methyl-1H-imidazo[4,5-c]quinolin-4-amine.

Part E

A suspension of N,N-bis(phenylmethyl)-1-ethoxymethyl-2-[(2-triphenylmethoxy)ethoxy]methyl-1H-imidazo[4,5-c]quinolin-4-amine (1.1 g, 1.5 mmole) in 6N hydrochloric acid (25 mL) was heated on a steam bath for 1.5 hours.

- 25 -

The reaction mixture was neutralized to pH 7 then extracted with methylene chloride. The extract was dried then concentrated under vacuum. The residue was purified by column chromatography (silica gel eluting with 10-50% ethyl acetate in hexanes v/v) to provide 0.5 g of N,N-bis(phenylmethyl)-2-(2-

5 hydroxyethoxy)methyl-1H-imidazo[4,5-c]quinolin-4-amine.

#### Part F

Triethylamine (0.17 mL, 1.25 mmole) was added to a solution of N,N-bis(phenylmethyl)-2-(2-hydroxyethoxy)methyl-1H-imidazo[4,5-c]quinolin-4-amine (0.5 g, 1.14 mmole) in methylene chloride (20 mL). Methanesulfonyl chloride (0.09  
10 mL, 1.14 mmole) was added and the reaction mixture was stirred at ambient temperature for 1 hour. The reaction mixture was diluted with methylene chloride, washed with water, dried over magnesium sulfate and then concentrated under vacuum to provide 0.6 g of N,N-bis(phenylmethyl)-2-(2-methylsulfonyloxyethoxy)methyl-1H-imidazo[4,5-c]quinolin-4-amine.

#### Part G

Excess potassium carbonate and excess sodium iodide were added to a solution of N,N-bis(phenylmethyl)-2-(2-methylsulfonyloxyethoxy)methyl-1H-imidazo[4,5-c]quinolin-4-amine (0.6 g, 1.14 mmole) in acetone (200 mL). The reaction mixture was heated at reflux overnight then concentrated under vacuum.  
20 The residue was partitioned between methylene chloride (150 mL) and water (50 mL). The methylene chloride was separated, dried over magnesium sulfate and then concentrated under vacuum. The residue was purified by column chromatography (silica gel eluting with 10:10:80 v/v/v methylene chloride:ethyl acetate:hexanes) to provide 0.42 g of N,N-bis(phenylmethyl)-10,11-dihydro-8H-[1,4]-  
25 oxazino[4',3':1,2]imidazo[4,5-c]quinolin-6-amine as a white solid.

#### Part H

Palladium hydroxide on carbon (0.5 g) was added to a solution of N,N-bis(phenylmethyl)-10,11-dihydro-8H-[1,4]-oxazino[4',3':1,2]imidazo[4,5-c]quinolin-6-amine (0.4 g, 0.95 mmole) in formic acid (about 40 mL). The reaction  
30 mixture was heated at reflux for 6 days then diluted with methanol and filtered

- 26 -

through a layer of Celite<sup>TM</sup> filter agent. The filtrate was made basic with ammonium hydroxide then concentrated under vacuum. The residue was taken up in a mixture of methanol and methylene chloride. Silica gel was added and the resulting mixture was concentrated under vacuum. The solid was placed on a column and eluted with  
5 2-5% methanol in methylene chloride to provide a white solid. The nuclear magnetic resonance spectra was consistent with the formate salt of the desired product. The salt was taken up in 5% hydrochloric acid and heated on a steam bath for 30 minutes. The mixture was made basic with 10% sodium hydroxide. The resulting precipitate was isolated by filtration, washed with water and dried under  
10 vacuum to provide 75 mg of 10,11-dihydro-8H-[1,4]-oxazino[4',3':1,2]-imidazo[4,5-c]quinolin-6-amine as a solid, m.p. 258-259°C. Analysis: Calculated for C<sub>13</sub>H<sub>12</sub>N<sub>4</sub>O: %C, 64.99; %H, 5.03; %N, 23.32; Found: %C, 64.61; %H, 4.88; %N, 23.18.

#### 15 INTERFERON (a) INDUCTION IN HUMAN CELLS

An in vitro human blood cell system was used to assess interferon induction by compounds of the invention. Activity is based on the measurement of interferon secreted into culture media. Interferon is measured by bioassay.

##### Blood Cell Preparation for Culture

20 Whole blood is collected by venipuncture into EDTA vacutainer tubes. Peripheral blood mononuclear cells (PBM's) are separated from whole blood by using either LeucoPREP<sup>TM</sup> Brand Cell Separation Tubes (available from Becton Dickinson) or Ficoll-Paque® solution (available from Pharmacia LKB Biotechnology Inc, Piscataway, NJ). The PBM's are suspended at  $1 \times 10^6$ /mL in  
25 RPMI 1640 media (available from GIBCO, Grand Island, NY) containing 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) and L-glutamine (1% penicillin-streptomycin solution added) with 10% heat inactivated (56°C for 30 minutes) autologous serum added. 200 µL portions of PBM suspension are added to 96 well (flat bottom) MicroTest III sterile tissue culture plates.

- 27 -

### Compound Preparation

The compounds are solubilized in ethanol, dimethyl sulfoxide or tissue culture water then diluted with tissue culture water, 0.01N sodium hydroxide or 0.01N hydrochloric acid (The choice of solvent will depend on the chemical characteristics of the compound being tested.). Ethanol or DMSO concentration should not exceed a final concentration of 1% for addition to the culture wells. Compounds are initially tested in a concentration range of from about 0.1  $\mu\text{g/mL}$  to about 5  $\mu\text{g/mL}$ . Compounds which show induction at a concentration of 0.5  $\mu\text{g/mL}$  are then tested in a wider concentration range.

### 10 Incubation

The solution of test compound is added in a volume (less than or equal to 50  $\mu\text{L}$ ) to the wells containing 200  $\mu\text{L}$  of diluted whole blood or of PBM's in media. Solvent and/or media is added to control wells (wells with no test compound) and as needed to adjust the final volume of each well to 250  $\mu\text{L}$ . The plates are covered with plastic lids, vortexed gently and then incubated for 48 hours at 37°C with a 5% carbon dioxide atmosphere.

### Separation

Following incubation, the plates are covered with parafilm and then centrifuged at 1000 rpm for 10 to 15 minutes at 4°C in a Damon IEC Model CRU-5000 centrifuge. Media (about 200  $\mu\text{L}$ ) is removed from 4 to 8 wells and pooled into 2 mL sterile freezing vials. Samples are maintained at -70°C until analysis.

### Interferon Analysis/Calculation

Interferon is determined by bioassay using A549 human lung carcinoma cells challenged with encephalomyocarditis. The details of the bioassay method have been described by G. L. Brennan and L. H. Kronenberg in "Automated Bioassay of Interferons in Micro-test Plates", Biotechniques, June/July, 78, 1983, incorporated herein by reference. Briefly stated the method is as follows: interferon dilutions and A549 cells are incubated at 37°C for 12 to 24 hours. The incubated cells are infected with an inoculum of encephalomyocarditis virus. The infected cells are incubated for an additional period at 37°C before quantifying for viral cytopathic

- 28 -

effect. The viral cytopathic effect is quantified by staining followed by spectrophotometric absorbance measurements. Results are expressed as alpha reference units/mL based on the value obtained for NIH HU IF-L standard. The interferon was identified as essentially all interferon alpha by testing in checkerboard  
5 neutralization assays against rabbit anti-human interferon (beta) and goat anti-human interferon (alpha) using A549 cell monolayers challenged with encephalomyocarditis virus. Results are shown in the table below wherein the absence of an entry indicates that the compound was not tested at that particular dose concentration.

Interferon (a) Induction in Human Cells									
Compound of Example	a Reference Units/mL								
	Dose Concentration (mg/mL)								
	0.01	0.05	0.10	0.50	1.0	5.0			
1	2	5	320	1000	370	46			
2	--	--	4	50	66	7			
3	--	--	4	100	130	32			
4	5	510	1200	160	190	380			
5	1	1	510	310	170	210			

- 30 -

## INTERFERON INDUCTION IN MICE

This test method was used to assess the ability of compounds of the invention to induce interferon biosynthesis in mice.

For each dose level being tested, three groups (three mice per group) of male mice (nonfasted) are dosed orally with compound. One hour later blood is collected from the retrobulbar plexus and pooled. The blood is centrifuged and serum collected and aliquoted. The serum samples are stored frozen at -70°C until analysis. This procedure is repeated at 2 hours post dose with the second group of mice and at four hours post dose with the third group of mice.

Samples are assayed as described above in connection with the analysis of interferon induction in human cells. The results are expressed in the table below as  $\alpha/\beta$  reference units/mL based on the value obtained for a mouse MU-1-IF standard.

Results are shown in the table below wherein results designated "<" a certain number indicate that interferon was not detectable in amounts above the lower sensitivity level of the assay.

Interferon Induction in Mice				
Compound of Example	Dose (mg/Kg)	Reference Units/mL		
		1 hr	2 hr	4 hr
1	0.3	<250	<250	<250
1	1.0	480	480	<250
1	3.0	480	1300	330
1	10.0	1600	4300	480
3	0.3	<380	<380	<380
3	1.0	<380	<380	<380



- 31 -

Interferon Induction in Mice				
Compound of Example	Dose (mg/Kg)	Reference Units/mL		
		1 hr	2 hr	4 hr
3	3.0	1100	820	<380
3	10.0	1500	2900	660
4	0.3	<520	520	<520
4	1.0	1100	1100	<520
4	3.0	2700	3500	<520
4	10.0	4700	11000	<520
5	0.3	<310	<310	<310
5	1.0	310	<310	<310
5	3.0	1100	1200	310
5	10.0	1700	2100	630

#### INDIRECT IN-VITRO ANTIVIRAL ACTIVITY

The test method described below demonstrates the ability of compounds of  
 5 the invention to inhibit the progress of viral infection.

Whole blood is collected by venipuncture into EDTA vacutainer tubes.  
 Peripheral blood mononuclear cells (PBM's) are isolated using Ficoll-Paque®  
 solution (available from Pharmacia LKB Biotechnology Inc., Piscataway, NJ). The  
 PBM's are washed with phosphate buffer saline then diluted with RPMI 1640  
 10 medium (available from GIBCO, Grand Island, New York) and 10% fetal bovine  
 serum to obtain a final concentration of  $2.5 \times 10^6$  cells/mL. One mL portions of

- 32 -

PBM's in medium are placed in 15 mL polypropylene tubes. The test compound is dissolved in dimethyl sulfoxide then diluted with RPMI 1640 medium. The solution of test compound is added to the tubes containing the PBM's to give final concentrations ranging from 0.05  $\mu\text{g/mL}$  to 1.0  $\mu\text{g/mL}$ . Control tubes do not

5 receive any test compound. The tubes are then incubated for 24 hours at 37°C with a 5% carbon dioxide atmosphere. Following incubation the tubes are centrifuged at 400 xg for 5 minutes. The supernatant is removed. The PBM's are brought up in 100  $\mu\text{L}$  of RPMI 1640 medium and then infected with a 100  $\mu\text{L}$  containing  $10^5$  tissue culture 50% infectious doses of vesicular stomatitis virus (VSV). The tubes

10 are incubated for 30 minutes at 37°C to allow virus adsorption. One mL of RPMI 1640 medium is added to each tube and the tubes are incubated for 48 hours at 37°C. The tubes are frozen then thawed to lyse the cells. The tubes are centrifuged at 400 xg for 5 minutes to remove cellular debris then the supernatant is assayed by serial tenfold dilutions on Vero cells in 96 well microtiter plates. The infected cells

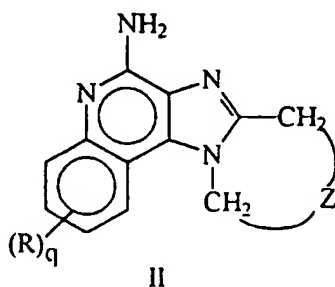
15 are incubated for 24 hours at 37°C before quantifying for viral cytopathic effect. The viral cytopathic effect is quantified by staining with 0.05% crystal violet. Results are presented as VSV inhibition, defined as the  $\log_{10}$  (control VSV yield/experimental VSV yield). Results are shown in the table below wherein the absence of an entry indicates that the compound was not tested at that particular

20 concentration. Control tubes have a value of 0.

In-vitro Antiviral Activity				
Compound of Example	VSV Yield Inhibition			
	Dose Concentration ( $\mu\text{g/mL}$ )			
	0.05	0.1	0.5	1.0
1	8.0	8.0	8.0	--
2	--	2.0	4.0	5.0

The Claimed Invention Is:

1. A compound of Formula II



wherein Z is selected from the group consisting of:

$-(CH_2)_n-$  wherein n is 1 to 4;

15  $-(CH_2)_a-C(R_1R_2)(CH_2)_b-$ , wherein a and b are integers and a+b is 0 to 3,  $R_1$  is hydrogen or alkyl of 1 to 4 carbon atoms, and  $R_2$  is selected from the group consisting of alkyl of 1 to 4 carbon atoms, hydroxy,  $-OR_3$  wherein  $R_3$  is alkyl of 1 to 4 carbon atoms, and  $-NR_4R'_4$  wherein  $R_4$  and  $R'_4$  are independently hydrogen or alkyl of 1 to 4 carbon atoms; and

20  $-(CH_2)_a-(Y)-(CH_2)_b-$  wherein a and b are integers and a+b is 0 to 3, and Y is O, S, or  $-NR_5-$  wherein  $R_5$  is hydrogen or alkyl of 1 to 4 carbon atoms;

and wherein q is 0 or 1 and R is selected from the group consisting of alkyl of 1 to 4 carbon atoms, alkoxy of 1 to 4 carbon atoms, and halogen,

and pharmaceutically acceptable salts thereof.

25

2. A compound according to Claim 1, wherein Z is  $-(CH_2)_n-$  and n is 1 to 3, and pharmaceutically acceptable salts thereof.

3. A compound according to Claim 1 wherein Z is  $-(CH_2)_a-$   
 $C(R_1R_2)(CH_2)_b-$  wherein a and b are integers and a+b is 0 to 3,  $R_1$  is hydrogen or  
alkyl of 1 to 4 carbon atoms, and  $R_2$  is selected from the group consisting of alkyl  
of 1 to 4 carbon atoms, hydroxy,  $-OR_3$  wherein  $R_3$  is alkyl of 1 to 4 carbon atoms,  
5 and  $-NR_4R'_4$  wherein  $R_4$  and  $R'_4$  are independently hydrogen or alkyl of 1 to 4  
carbon atoms, and pharmaceutically acceptable salts thereof.

4. A compound according to Claim 3, wherein  $R_1$  is hydrogen and  $R_2$  is  
alkyl of 1 to 4 carbon atoms, and pharmaceutically acceptable salts thereof.

10

5. A compound according to Claim 1, wherein Z is  $-(CH_2)_a-(Y)-(CH_2)_b-$   
wherein a and b are integers and a+b is 0 to 3, and Y is O, S, or  $-NR_5-$  wherein  $R_5$   
is hydrogen or alkyl of 1 to 4 carbon atoms, and pharmaceutically acceptable salts  
thereof.

15

6. A compound according to Claim 5, wherein Y is O, and  
pharmaceutically acceptable salts thereof.

7. A compound according to Claim 6, wherein a+b is 1, and  
20 pharmaceutically acceptable salts thereof.

8. A compound according to Claim 1, selected from the group consisting of:  
8,9,10,11-tetrahydropyrido[1',2':1,2]  
imidazo[4,5-c]quinolin-6-amine,  
25 10-methyl-8,9,10,11-tetrahydropyrido[1',2':1,2]  
imidazo[4,5-c]quinolin-6-amine,  
8H-9,10,11,12-tetrahydrohexamethylene-imino[1',2':1,2]imidazo[4,5-  
c]quinolin-6-amine,  
9,10-dihydro-8H-pyrrolo[1',2':1,2]imidazo[4,5-c]quinolin-4-amine,  
30 10,11-dihydro-8H-[1,4]-oxazino[4',3':1,2]imidazo[4,5-c]quinolin-6-amine,

and pharmaceutically acceptable salts thereof.

9. A pharmaceutical formulation comprising: (i) a compound according to Claim 1 in an amount effective to induce interferon biosynthesis in an animal, and  
5 (ii) a pharmaceutically acceptable vehicle.

10. A method of inducing interferon biosynthesis in an animal, comprising the step of administering to said animal a compound according to Claim 1 in an amount effective to induce said interferon biosynthesis.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/15828

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D471/14 C07D498/14 A61K31/435 A61K31/55 C07D513/14  
 //(C07D471/14,235:00,221:00,221:00),(C07D471/14,235:00,223:00,  
 221:00),(C07D471/14,235:00,221:00,209:00),(C07D498/14,265:00,235:00,  
 221:00)  
 According to International Patent Classification (IPC) or to both national classification and IPC 221:00)

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 385 630 (RIKER) 5 September 1990 cited in the application see claims 1,10,13	1,9
A	WO,A,92 15582 (MINNESOTA MINING) 17 September 1992 cited in the application see claims 1,41,44	1,9

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*&\* document member of the same patent family

Date of the actual completion of the international search

9 April 1996

Date of mailing of the international search report

17. 04. 96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+ 31-70) 340-3016

Authorized officer

Alfaro Faus, I

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/15828

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Although claim 10 is directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 95/15828

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-385630	05-09-90	AU-B- 630921	12-11-92
		AU-B- 5005490	30-08-90
		CA-A- 2010430	27-08-90
		JP-A- 3027380	05-02-91
-----			
WO-A-9215582	17-09-92	AU-B- 658621	27-04-95
		AU-B- 1566992	06-10-92
		AU-B- 2715795	21-09-95
		CA-A- 2104782	02-09-92
		CZ-A- 9301788	18-10-95
		EP-A- 0582581	16-02-94
		HU-A- 67026	30-01-95
		IL-A- 101110	08-12-95
		JP-T- 6504789	02-06-94
		NO-A- 933069	01-11-93
		NZ-A- 241784	27-06-95
		US-A- 5389640	14-02-95
-----			